

(19)



Europäisches Patentamt

European Patent Office

Office européen des brevets



(11)

EP 0 430 517 B1

(12)

EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention
of the grant of the patent:
22.04.1998 Bulletin 1998/17

(51) Int Cl.⁶: **C08F 2/24, C08F 2/32,
G01N 33/545**

(21) Application number: **90312532.6**

(22) Date of filing: **16.11.1990**

(54) Biomosaic polymers and method for preparing same

Biomosaïsche Polymere und Verfahren zu deren Herstellung

Polymères biomosaïques et leur procédé de préparation

(84) Designated Contracting States:
BE DE FR GB IT NL SE

(30) Priority: **21.11.1989 US 439630**

(43) Date of publication of application:
05.06.1991 Bulletin 1991/23

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(56) References cited:
EP-A- 0 178 791
GB-A- 1 411 386
US-A- 3 793 445
US-A- 4 552 633
EP-A- 0 226 470
GB-A- 2 071 669
US-A- 4 322 311

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DescriptionField of the Invention

5 The present invention relates to biomosaic polymeric membranes having biologically active material bound at surfaces thereof and the method for preparing the same. The polymeric membrane is a porous membrane, and the biologically active material may be useful for biospecific reactions such as immunoassays, bioseparations, enzyme-catalyzed reactions and the like.

Background of the Invention

10 Special problems are presented in the biochemical reactions used to assay a specific composition in a mixture of materials, separate one material from another, or catalyze a reaction using an enzyme. Frequently, the chemicals or molecules useful for such assays, separations, or reactions are scarce and costly. If such biologically active materials are not attached to a support material by some means, the scarce and costly materials will be lost for further usage, repeat experiments, and the like.

Therefore, in the biochemistry arts, methods involving a sequence of processing steps have been developed to immobilize various biologically active materials to supports where the supports have been formed in the absence of biologically active materials. Generally, such methods of immobilization may be divided into the following categories: 20 covalent bonding or crosslinking of the biologically active material to a support; adsorption onto a support; or inclusion in a polymer matrix wherein the biologically active material is trapped or encapsulated in the lattice of the polymer. (A. Hoffman, Radiat. Phys. Chem. 18(1,2), 323-342 (1981)).

However, depending upon the application, immobilization by covalent bonding or crosslinking may damage the biologically active material to the extent that its activity is reduced or lost. (K. Buchholz et al., Meth. Enzymol. 135, 3-30 (1987)). Even if the biologically active material retains its biological activity, experience has shown in production of covalently bonded immobilized materials that the distribution of biologically active materials may not be uniform.

Adsorption of such materials to a surface often results in the loss of activity. Further, adsorption is often a reversible process such that the biologically active material can be lost from the support by equilibration with the reaction media which may cause undesired contamination of the reaction products. (J. Andrade in "Surface and Interfacial Aspects of Biomedical Polymers, Volume II Protein Adsorption", Chapter 1, J. Andrade Ed., Plenum Press, New York (1985)).

30 Inclusion of the biologically active material by entrapment or encapsulation has previously been a preferred method for enzymes since the method relies upon the physical trapping of a large molecule in a polymer matrix from which it is very slow to escape. (A. Hoffman, referenced above). For materials which interact with the entrapped molecule and which are small enough to diffuse through this matrix, these methods are satisfactory, but for large reaction materials such as large proteins, the matrix prevents enzyme reactions.

Further, entrapment methods conventionally employ gel matrices formed by the polymerization of hydrophilic monomers and crosslinkers to form hydrogels. (United States Patent Number 3,788,950 and K. O'Driscoll et al., Biotech. Bioeng. 14, 849-850 (1972)). These hydrogels are typically mechanically weak and may not be able to withstand the rigors of repeated usage without breaking up. Thus, the biologically active material and its matrix may be lost during the assay, separation, or reaction.

Encapsulations have been performed using gamma irradiation to polymerize hydrophilic monomers in order to trap enzymes, but such radiation polymerization has required the freezing of the enzyme/monomer mixture to encapsulate the enzymes therein. (I. Kaetsu et al., Radiat. Phys. Chem. 14, 595-602 (1979)). Thus, the immobilization of an enzyme in a hydrophilic matrix by this process requires excessive energy. Further, using gamma radiation of high 45 dosages could destroy the biological activity of the enzyme. (E. Galas et al., Radiochem. Radioanal. Let. 43(6), 355-362 (1980)).

Hydrophobic monomers have also been used to entrap enzymes by polymerization of frozen mixtures or dispersions. These processes result in the preparation of beads as the hydrophobic monomers separate into droplets as the water freezes. (I. Kaetsu et al., Biotech. Bioeng. 21, 863-873 (1979)).

50 Unfortunately, the prior methods of encapsulation or entrapment of biologically active materials do not allow the immobilized species to bind or react with large molecules while retaining the species within the matrix. Encapsulation or entrapment would not allow for the immobilized species to interact with both large and small molecules.

In other words, most of the methods for direct incorporation of biologically active materials into the support relate to supports such as beads, gels, and particles.

55 Immobilization of biologically active materials onto the surfaces of membrane supports is known, but the active materials are introduced after membrane formation. (European Patent Office, Publication 0 294 186). This can lead to problems of leakage of the biologically active material, or non-uniform distribution of the biologically active material on the support, or require additional processing steps. Membrane supports are particularly desirable for bioseparations,

enzyme-catalyzed reactions and the like.

Conventional processes have also been developed for the preparation of emulsions in the presence of biologically active materials. Such emulsions may be either water in oil (W/O) or oil in water (O/W) or other combinations of hydrophilic/hydrophobic liquids. (United States Patent Number 4,774,178).

With the use of some surfactants, biologically active materials may be dispersed into an emulsion which qualifies as a microemulsion because of the microscopic extent of dispersion of the oil and water constituents. (P.L. Luisi, *Angew. Chem. Int. Ed. Engl.* 24(6), 439-528 (1985)). When the oil and water components of the microemulsion are so dispersed that it is not possible to characterize the microemulsion as either a water in oil microemulsion or an oil in water microemulsion, a bicontinuous microemulsion is recognized. (L.E. Scriven, *Nature* 263, 123 (1976)).

Thus, the water and oil components of the microemulsion are so intermixed that both components are intimately mixed in continuous contact with like material. A suitable analogy for a bicontinuous microemulsion is a water saturated sponge. Both the water and the sponge components are intimately mixed but each is also in continuous contact with its like material.

Previously processes have used microemulsions to prepare polymers and beads. (J.O. Stoffer et al., *J. Dispersion Sci. and Technol.* 1(4), 393-412 (1980) and United States Patent Number 4,521,317). However, the polymerization of microemulsions containing biologically active materials has only been used to prepare nanometer-sized particles. (United States Patent Number 4,021,364). Films and membranes, most desired for biological reactions such as assays, separations and enzyme-catalysis, have not been produced by microemulsion techniques.

US-A-4,322,311 discloses a process for producing microcapsules of controlled porosity containing a chemically active core material. A core material such as a biologically active material and a first monomer in aqueous solution are emulsified in a hydrophobic solvent. A monomer complementary to the first and soluble in the continuous, hydrophobic phase of the emulsion is added to initiate interfacial polymerization about the aqueous droplets. During the course of the reaction, the affinity of the continuous phase for the first monomer is varied by adding a solvent to the continuous phase to vary its polarity. The continuous phase may be relatively nonpolar at the outset and a polar solvent may be added to increase its affinity for the first monomer, or it may be relatively polar at the outset and a nonpolar (or less polar) solvent may be added. It is disclosed that that technique allows production of microcapsules having a selected pore size so that, for example, encapsulated material cannot escape, yet lower molecular weight materials can diffuse through the capsule wall.

Thus, what is needed is a polymeric membrane formed having biologically active or reactive sites at its surfaces in order to enable biochemical usage. What is also needed is a method of preparing the polymeric membrane using a low cost emulsion method which does not harm the bioreactivity of the biologically active material.

Summary of the Invention

The present invention overcomes the problems in the art by providing a porous, biomosaic, polymeric membrane said membrane comprising at least a minimally useful amount of at least one biologically active material irreversibly bound at surfaces of said membrane, wherein said membrane has pores from 0.01 micrometers to 10 micrometers, and wherein said membrane is a polymer formed from microemulsion of at least one hydrophobic polymerizable monomer polymerized in the presence of said biologically active material.

In another embodiment there is provided the use of the above membrane for immunoassay as an enzyme reactor, as a bioseparator, or combinations thereof.

In yet another embodiment there is provided a process for preparing a porous, biomosaic, polymeric membrane wherein said polymer is prepared by polymerization at no lower than ambient temperature of a water-in-oil or a bicontinuous microemulsion of a hydrophobic polymerizable monomer, a biologically active material, at least one hydrophilic liquid, and at least one surface active agent;

wherein polymerization comprises mixing said microemulsion, dispensing said microemulsion to form the polymeric membrane, and polymerizing said microemulsion at ambient temperature and with high energy radiation to polymerize said hydrophobic polymerizable monomer to form a biomosaic porous polymeric membrane having said biologically active material bound at surfaces of said polymer.

The biomosaic polymer useful in the invention is prepared as foreshadowed above by polymerizing a specific liquid/liquid dispersion (ie. a microemulsion) which contains a significant amount of surface active agent to assist in the development of a sufficiently stable microemulsion and to assure binding of the biologically active material at the surfaces of the biomosaic polymer.

A "biomosaic polymer" means a polymer having at least one biologically active material bound at surfaces of the polymer formed from a polymerized microemulsion of at least one hydrophobic polymerizable monomer polymerized in the presence of such biologically active material. Because biologically active material is present during polymerization, the bioreactive sites are created by such one or more biologically active materials bound at surfaces of the polymer, wherever such surfaces may be located in the structure of the polymer so formed. The biologically active material

present in the mass of the biomosaic polymer is congregated at surfaces of such biomosaic polymer where such biologically active material may be biochemically useful.

"Surfaces" means any surface or surfaces of the biomosaic polymer formed, ie. the outer surface and the surfaces of pores of the porous membrane.

5 "Bound" means that biologically active material is chemically or physically combined with the hydrophobic polymerizable monomer at surfaces formed during polymerization such that at least a minimally useful amount of biologically active or reactive sites is exposed.

"Congregated" means that the amount of biologically active material present during polymerization and combining with the hydrophobic polymerizable monomer is overwhelmingly located at surfaces of the biomosaic polymer.

10 Compared with previously known materials, biologically active material in the biomosaic polymer useful in the present invention is neither physically entrapped within the polymer interstices nor merely subsequently attached at the surface of a polymer previously prepared.

While not intending to be limited to a particular theory, it is believed that biologically active material present during the polymerization of the polymerizable monomer, as facilitated by the surface active agent, becomes irreversibly part of surfaces of the polymer so formed. With some portion or portions of the molecule(s) of biologically active material contributing to the surface formation of the polymer, that portion or portions of the molecule(s) of biologically active material become(s) bound at surfaces and the remaining portions of the bound molecule(s) of biologically active material not bound. So bound as a part of exposed surfaces of the biomosaic polymer and forming a mosaic of bioreactivity at such surfaces, biologically active material is like cilia prepared to interact with that which passes by.

20 The unexpectedly large amount of biologically active material bound at surfaces of the biomosaic polymer can be expressed in terms of mass/surface area. It has been found according to the present invention that from as little protein as is minimally useful to as much protein as about $70 \mu\text{g}/\text{cm}^2$ of a membrane of such biomosaic polymer can be bound at surfaces of the polymer.

25 The unexpectedly large amount of biologically active material bound at surfaces of the biomosaic polymer can also be expressed in terms of the efficiency of binding of available protein. It has been found according to the present invention that between 10 percent and 50 percent of the protein available was bound at surfaces of the biomosaic polymer. It is believed that between 1 percent and 90 percent could be so bound.

30 The unexpectedly large amount of biologically active material bound at surfaces of the biomosaic polymer can also be expressed in terms of mass/mass comparisons with the biomosaic polymer. Depending on the weight fraction ratio of biologically active material to polymerizable monomer present for polymerization, it has been found according to the present invention that the mass/mass comparison can be between as little as minimally useful to as much as 20 mg/g of biomosaic polymer.

35 Finally, the unexpectedly large amount of / biologically active material bound at surfaces of the biomosaic polymer can also be expressed in terms of a percentage of surface area having biologically active material so bound. It has been found according to the present invention that as little protein as is minimally useful to as much as about 35 percent of the surface area of the biomosaic polymer had biologically active material so bound. It is possible that with as much as 90 percent efficiency of binding as much as about 85 percent of the surface area of the biomosaic polymer could have biologically active material so bound.

40 It should be understood that the present invention contemplates the binding of one or more kinds of biologically active material with one or more polymerizable monomers and could even exceed the ranges presently known.

At least a three component system is used to prepare the biomosaic polymer having biologically active material bound at the polymer: hydrophobic polymerizable monomer, biologically active material, and surface active agent. These three components are in a microemulsion of hydrophobic liquid/hydrophilic liquid. At ambient temperature, the monomer is polymerized in the presence of biologically active material and surface active agent.

45 In one embodiment of the invention, the microemulsion used to prepare the biomosaic polymer can be a two liquid system having multiple components in immiscible liquids: a hydrophobic polymerizable monomer in or as one liquid, and a hydrophilic liquid immiscible with the hydrophobic polymerizable monomer as the other liquid. The biologically active material may be in either liquid or both liquids, although preferably in the hydrophilic liquid prior to mixing to form the microemulsion. The surface active agent may be in either liquid or both liquids, although preferably in the hydrophobic liquid prior to mixing to form the microemulsion.

50 The present invention also solves the problems inherent in the use of excessive energy for a polymerization process by conducting such polymerization at ambient temperatures.

55 The present invention also solves the problems of biochemical reactivity for the assay, bioseparation or catalyzed reaction, by forming a polymer having a biologically active material bound at surfaces of the biomosaic polymer, thereby permitting facile interaction with both large and small molecules without a loss of that costly and scarce material during each biochemical interaction.

The present invention also solves the problems inherent in subsequent interaction of a biologically active material with a previously formed polymer.

The present invention will become apparent in view of the following description of the invention.

Brief Description of the Drawing

- 5 Fig. 1 is a graph showing a phase diagram for one embodiment of the present invention described in Example 1 below;
 Fig. 2 is a graph showing the results of Example 17 described below; and
 Fig. 3 is a graph showing the results of Example 18 described below.

Embodiments of the Invention

EMULSION COMPONENTS AND PHASES

15 The biomosaic polymer having biologically active materials bound at surfaces thereof useful in the present invention is achieved by the preparation of a hydrophobic liquid/hydrophilic liquid microemulsion having three significant components: at least one polymerizable monomer, at least one surface active agent, and at least one biologically active material. Desired forms of microemulsions are those which form spontaneously and have good thermodynamic stability.

Preferably, the hydrophilic liquid is water or any other hydrophilic solvent in which the biologically active material may be stable. Preferably, the hydrophobic liquid is the hydrophobic polymerizable monomer capable of polymerization
 20 from a microemulsion.

The amounts of the three components described above used in the microemulsion depend on the desired properties of the biomosaic polymer. Proper amounts can be derived from phase diagrams involving all three components or their solvents. Phase diagrams and the various structures which may be adopted by the components prior to polymerization have been described in numerous publications including "Microemulsions: Theory and Practice", ed. L.
 25 M. Prince, Academic Press, NY, 1977, pp. 133-148, and in "Micellization, Solubilization and Microemulsions", Volumes I and II, ed. Mittal, K.L., Plenum, New York, 1977, pp 45-53 and references therein. One skilled in the art may be able to use such phase diagrams to determine the proper amounts of the components. The phase diagram will change depending upon the components used.

Non-limiting examples of microemulsions include W/O microemulsions of higher water and surface active agent
 30 content for porous structures such as membranes.

For the purposes of the present invention to identify the stable clear W/O region, Fig. 1 illustrates a phase diagram comprising at one apex, polymerizable hydrophobic monomer, at the second apex surface active agent, and at the third apex, the hydrophilic liquid, water, into which biologically active material may be placed. Depending on the respective weight fractions of all components, the dispersion ranges from a cloudy emulsion to a clear microemulsion.

35 While not being bound to any particular theory, in a preferred embodiment of the invention, polymerizable microemulsions comprising polymerizable monomer, surface active agent, and biologically active material in the hydrophilic liquid are believed to have sufficient surface interaction as to provide droplets of hydrophilic liquid containing biologically active material having a diameter in the range of from 5 nm to 80 nm and the hydrophobic polymerizable monomer and surface active agent as the continuous medium. As polymerization proceeds, phase separation of the biomosaic
 40 polymer occurs during formation. That formation is believed to be facilitated by the intermediate production of bicontinuous or other characteristics as the microemulsion composition changes.

POLYMERIZABLE MONOMER

45 The polymerizable monomer is present in the "oil" component of the emulsion and is therefore hydrophobic. The polymerizable monomer may be present in the "oil" component in bulk, in dispersion, or in solution. "Hydrophobic" means that the polymerizable monomer has a solubility in water of less than 1 part in 100 parts (weight/weight) of water. However, in the presence of the surface active agent this hydrophobic monomer may become so dispersed as to form a microemulsion in the presence of hydrophilic liquids. By contrast, "hydrophilic" means a water-miscible compound that is capable of forming an emulsion or microemulsion with the "oil" component.

"Monomer" for purposes of this invention means at least one monomer, prepolymer, oligomer, or combinations thereof, capable of forming a microemulsion in the presence of a surface active agent and a hydrophilic liquid. The monomer must be polymerizable by methods known in the art, for example, such as described in Volume 4 of "Encyclopedia of Polymer Science and Technology", ed., H.F. Mark et al., Interscience, New York, 1966, pp 331-414. Desirably, the polymerizable monomer is polymerizable by irradiation, e.g., using high energy electromagnetic radiation.
 55 The oil component may also contain a photoinitiator to facilitate polymerization of the polymerizable monomer.

Generally, hydrophobic polymerizable monomers of the present invention are addition-polymerizable unsaturated organic compounds having at least one double bond between two carbon atoms. Desirably, at least one of these carbon

atoms also has bonded thereto a carboxyl or carboxylate ester functionality. Such hydrophobic polymerizable monomers are well known in the radiation-induced polymer coating art, and lists of suitable monomers can be found in publications by J.J. Wildi ("Surface Coatings, Australia", June 1986, p.19); R. Holman ("U.V. and E.B. Curing Formulations for Printing Inks, Coatings and Paints", SITA Technology, London, 1984, pp. 49-60); and United States Patent Number 4,466,931.

Of the compositions described above, desirably hydrophobic polymerizable monomers terminated by one or more acrylate groups are useful in the preparation of the polymer in accordance with the present invention. Non-limiting examples include monofunctional acrylates and methacrylates, such as isooctyl acrylate (IOA), isobornyl acrylate (IBA), 2-ethylhexyl acrylate (2-EHA), ethylenediglycol acrylate (EDGA), and ethyltriglycol methacrylate (ETGMA); difunctional acrylates and methacrylates, such as 1,6-hexanediol diacrylate (HDDA), tetraethyleneglycol diacrylate (TEGDA), tripropyleneglycol diacrylate (TPGDA), and propoxylated glycol diacrylate (POGDA); trifunctional acrylates and methacrylates, such as pentaerythritol triacrylate (PETA), and trimethylolpropane triacrylate (TMPTA); and tetrafunctional acrylates and methacrylates, such as pentaerythritol tetraacrylate (PETTA), and di-trimethylolpropane tetraacrylate (DTMPTTA).

Preferably, for the preparation of a flexible, non-fragile polymer structure, a hydrophobic polymerizable monomer having a monofunctional acrylate group, such as isobornyl acrylate (IBA), may be used in combination with one or more polymerizable monomers having multifunctional acrylate groups, such as propoxylated glycol diacrylate (POGDA), in a range of weight percentages of from 5 percent to 95 percent of the total weight of all of the hydrophobic polymerizable monomers.

For a preferred practice of the present invention, it is preferable to include a photoinitiator in the hydrophobic polymerizable monomer. A photoinitiator is a molecule which can absorb radiant energy and undergo a chemical process which results in reactive intermediates such as free radicals capable of initiating the polymerization of the hydrophobic polymerizable monomer. Many photoinitiators are known in the art and have been described in part in the publications by Wildi and Holman referenced above. Non-limiting examples include benzoin and its derivatives, benzil ketals, acetophenone derivatives, benzophenones such as Michler's ketone, and anthraquinones. Preferred amounts of the photoinitiator in the hydrophobic polymerizable monomers range from 0.1 to 10 weight percent of the hydrophobic polymerizable monomer.

The hydrophobic polymerizable monomers of the present invention are present in an amount of from 1 percent to 97 percent of the weight of the microemulsion. Desirably, the hydrophobic polymerizable monomers are in an amount of from 25 percent to 80 percent of the total weight of the microemulsion to obtain porous polymer structures. Preferably, when forming a porous membrane, the amount of the hydrophobic polymerizable monomer in the emulsion is from 35 percent to 65 percent of the weight of the emulsion, in order to assure polymer integrity.

SURFACE ACTIVE AGENT

"Surface active agent" means a material which may be nonionic, ionic, or zwitterionic. Ionic includes both cationic and anionic. Generally, the interaction of a protein with a surface is prevented by the presence of a surface active agent. (See, e.g., C. Tanford in the "Hydrophobic Effect", Chapters 12, 16, and 19, J. Wiley and Sons, First Edition (1973)). Unexpectedly, such surface active agents used in accordance with the present invention actually appear to enhance the ability of a polymerizable monomer at the time of polymerization to bind unexpectedly large amounts of the biologically active material at surfaces of the polymer formed. The biologically active material is bound at surfaces of the polymer in such amounts only in the presence of the surface active agent at the time of polymerization.

Surface active agents useful in accordance with the present invention are molecules which consist of a hydrophobic group and a hydrophilic group. One or more surface active agents may be used in accordance with present invention. Many examples and types are known such as described by M.J. Rosen in "Surfactants and Interfacial Phenomena" (Wiley-Interscience, New York, 1978) at pp. 1-25. Commercially available surface active agents are listed in "McCutcheon's Detergents and Emulsifiers" (McCutcheon Division, MC Publishing Co., Glen Rock, N.J., published annually through at least 1989).

Non-limiting useful examples of anionic surface active agents include salts of carboxylic acids preferably having a carbon chain length of from 8 to 22 atoms, sulfonic acids, sulfuric acid esters, and phosphoric and polyphosphoric acid esters.

Non-limiting examples of cationic surface active agents include quaternary ammonium salts, and other salts of amines, diamines, polyamines, amine oxides, or polyoxyethylenated amines; (all such amines preferably having a carbon chain length from 8 to 22 atoms).

Non-limiting examples of nonionic surface active agents include polyoxyethylenated-alkyl phenols, polyoxyethylenated-straight chain alcohols, polyoxyethylenated-polyoxypropylene glycols, and carboxylic acid esters preferably having a carbon chain length from 8 to 22 atoms.

Non-limiting examples of zwitterionic surface active agents include beta-N-alkylaminopropionic acids, N-alkyl-beta-

iminodipropionic acids, imidazoline carboxylates, N-alkylbetaines, sulfobetaines, and sultaines.

Preferred surface active agents are the nonionic surface active agents, in order to minimize degradation of bioactivity of the biologically active material. The actual choice of the surface active agent will depend upon the selection of the other components for the micro emulsion

The amount of the surface active agent present in the microemulsion will depend upon the solubility of the surface active agent in the hydrophobic polymerizable monomer constituting the "oil" component of the emulsion.

Generally, for the formation of membranes, the weight of surface active agent in the microemulsion will range from 2 percent to 60 percent, in order to assure binding of biologically active material at the surfaces of the polymer. Desirably, in order to assure the formation of a porous polymer membrane, the weight of surface active agent in the microemulsion is from 10 percent to 50 percent. Preferably, to yield a desirable porous polymer having biologically active material bound at surfaces of the polymer, the weight of the surface active agent in the microemulsion is from 20 percent to 50 percent.

BIOLOGICALLY ACTIVE MATERIAL

"Biologically active material" means a substance which is biochemically, immunochemically, physiologically, or pharmaceutically active or reactive. Biologically active material includes at least one or more of the following: biochemical compounds (such as amino acids, carbohydrates, lipids, nucleic acids, proteins, and other biochemicals and substances which may complex or interact with biochemical compounds), such biochemical compounds biologically functioning as antibodies, antigenic substances, enzymes, co-factors, inhibitors, lectins, hormones, receptors, coagulation factors, growth enhancers, histones, vitamins, drugs, cell surface markers, herbicides, and pesticides, among others known to those skilled in the art. Of the group of biologically active materials described, proteins are frequently desired by those skilled in the art as materials to be immobilized.

Any one or more of the possible biologically active materials may be useful in accordance with the present invention.

Non-limiting examples include an allergy diagnosis assay using a membrane having an extract of perennial ryegrass pollen an affinity separation reaction using Protein A for separation of immunoglobulin, or an enzyme-catalyzed reaction using esterase for ester hydrolysis.

Biologically active material can be introduced into the microemulsion at any time before polymerization. Preferably, the biologically active material is included in the hydrophilic component prior to formation of the microemulsion. The hydrophilic component is preferably water. The amount of biologically active material bound at surfaces of the polymer prepared by the microemulsion depends on the concentration of biologically active material in the hydrophilic component.

The concentration of biologically active material in the hydrophilic component can be as little as is minimally useful and up to saturation level. Desirably, biologically active material is present in the hydrophilic component to about 10 weight percent of the total weight of the hydrophilic component to minimize precipitation when included in the microemulsion. Preferably, up to 5 weight percent of biologically active material can be used in the hydrophilic component, both to produce a sufficiently stable microemulsion for emulsion processing and to provide subsequently useful bioactivity levels.

The amount of hydrophilic component containing biologically active material in the microemulsion can range from 1 percent to 97 percent by weight of the microemulsion. Desirably, to produce porous structures from microemulsions, the amount can range from 5 percent to 50 percent by weight. Preferably, to ensure good physical handling of the components in the microemulsion, the amount can range from 10 percent to 40 percent by weight.

PREPARATION OF THE POLYMER

The polymerizable microemulsions of the present invention are preferably prepared by dispersing or dissolving biologically active material in a hydrophilic solution and, in a separate vessel, mixing the polymerizable monomer and the surface active agent together. The biologically active material in the aqueous solution and the mixture of the surface active agent and the polymerizable monomer are then combined and vigorously mixed together as necessary to give the microemulsion.

Microemulsions are well known to be easily formed with minimal agitation and to be thermodynamically stable. They can be prepared by simply shaking the components together in a stoppered flask for about 10 to 30 seconds at ambient temperatures and pressures. Continuous agitation may be necessary for the less stable emulsions.

The thoroughly mixed microemulsion is dispensed, e.g., by extrusion, prilling or spraying into a receiving vessel which may be but need not serve as a mold for the structure the polymer will take upon polymerization. The dispensed microemulsion is then polymerized by any of the known methods referenced above. Desirably, the dispensed microemulsion can be polymerized by irradiation with high energy radiation such as ionizing particle radiation (e.g., electron beam emissions) or ionizing electromagnetic irradiation (e.g., gamma irradiation.) Preferably, the radiation is near ultra-

violet, ultra-violet, or visible light, and most preferably is ultra-violet light.

Polymerization of the polymerizable monomer in the microemulsion in the presence of the surface active agent in order to form a biomosaic polymer having biologically active material bound at surfaces thereof depends on the components used and their weight fractions in the mixture. Desirably, the polymerization conditions are chosen to minimize temperature rises, which could alter the biological activity of the biologically active material. Yet such conditions must allow substantially complete polymerization of the polymerizable monomer as to form the desired biomosaic polymer. For example, the polymerization may use one or more irradiations.

The temperature of the polymerization process may be ambient. There is no need for depressed temperatures and generally no need for elevated temperatures for the polymerization process of the present invention. Ambient temperature means the room temperature conditions of laboratories or production facilities throughout temperate climates in the world and therefore ranges from 10°C to 35°C.

After polymerization is substantially complete, a biomosaic polymer structure is formed having the biologically active material bound at surfaces of the structure, including its outer surfaces and any porous infrastructure surfaces. This structure can be washed, e.g., with solvents such as methanol and water to remove extraneous and unreacted materials.

The resulting washed polymer can be air dried and yields a white, flexible, porous biomosaic polymer suitable for use, e.g., reaction, separation, or assay. Optionally, coloring agents may be added to the emulsion to produce a tinted biomosaic polymer.

USEFULNESS OF THE INVENTION

The presence of surface active agent and biologically active material in the microemulsion at the time of polymerization of the polymerizable monomer allows the biologically active material to be bound at the same time as polymerization of the polymerizable monomer. Unlike entrapment or encapsulation techniques used previously, the biologically active material is believed to be a part of the polymer at its surfaces having at least a minimally useful amount of biologically active or reactive sites exposed. Unlike adsorption, covalent bonding or crosslinking, the biologically active material is bound at the time of the polymerization, not at some time subsequently when the processing techniques may be more costly, more damaging to the bioreactivity of the material, or yield fewer or non-uniformly spaced bioreactive sites on the surface of the previously formed substrate.

Use of the emulsion polymerization process at ambient temperatures and pressures, preferably using radiation polymerization techniques, avoids costly emulsion polymerization techniques at freezing temperatures and yields a greater amount of biologically active material at the surfaces of the biomosaic polymer.

Biomosaic polymers formed according to the methods of the present invention retain significant amounts of biologically active material at their surfaces.

Tests are known to measure activity of biologically active material, for example as reported by P.K. Smith et al. in "Measurement of Protein Using Bicinchoninic Acid" Analytical Biochemistry 150, 76-85 (1985).

In accordance with the present invention, of the constituents and reaction parameters described above, it is possible for one skilled in the art to prepare a biomosaic polymer to retain a biological activity of from 1 percent to 90 percent of the original amount of biologically active material in the microemulsion thus demonstrating the significant amount of biologically active material bound at surfaces of the biomosaic polymer. Likely, one skilled in the art can attain an efficiency of binding of biologically active material from 5 percent to 75 percent of the amount of biologically active material available in the microemulsion. Most likely, one skilled in the art can attain an efficiency of from 10 percent to 50 percent.

The biomosaic polymer may be polymerized in the form of a porous membrane. The surfaces of the structure are significantly reticular and have biologically active material bound thereat. It is possible to form membranes, for example, by extruding through a die or by coating on a permanent or temporary support, and immediately passing through an irradiation beam. The biomosaic polymer is in the form of a porous membrane with biologically active material bound at surfaces including the outer surfaces, pores throughout the depth of the membrane and pores into part of the depth of the membrane. The pores of the membrane are from 0.01 micrometers to 10 micrometers. Such porous structures significantly increase the surface area of the biomosaic polymer in order to facilitate uses such as assays, bioseparations, or catalyzed reactions.

In the form of a membrane, it is within the scope of the present invention to prepare multiple layers of membranes of biomosaic polymers having various biologically active materials bound at surfaces thereof thereby providing multiple functionalities or multiple selectivities for assays, bioseparations and catalyzed reactions.

The biomosaic polymers useful in the present invention are thereby available for a wide range of utility, including enzyme immunoassays, fluorescent immunoassays, chemiluminescent immunoassays, radio immunoassays, catalyzed reactions such as the removal of one of a chiral pair from a reaction mixture, the enzymic conversions, e.g., conversion of starch to glucose using amylase; and affinity separations such as removal of products and/or contami-

nants from mass culture fermentation/tissue culture reactions or from blood of patients with diseases such as cancer or autoimmune diseases.

It is also within the scope of this invention to have more than one type of biomosaic polymer having the same or different biologically active materials bound at its surfaces. It is also within the scope of the present invention to provide a combination of biomosaic polymers utilizing the same or different surface active agents. By utilizing the variety of reactivities of the principal components in the emulsion, a combination of bioreactivities of the resulting biomosaic polymer with biologically active material bound at its surfaces may be achieved.

While not intended to be limited, thereto or thereby, the following examples represent the practice and utility of the present invention for a variety of purposes of biological activity.

REFERENCE

Example 1

This example illustrates the determination of the desired microemulsion region of the phase diagram for one combination of polymerizable monomer acting as the "oil" component, surface active agent, and water useful to include biologically active material therein.

Into 9.5 g of a liquid hydrophobic polymerizable monomer (comprising 85 parts isobomyl acrylate (available from Rohm and Haas of Philadelphia, PA as product "QM589"), 10 parts propoxylated glycol diacrylate (Product "Photomer 4083" available from Henkel Corporation, formerly Diamond Shamrock Chemical Corporation, of Morristown, NJ) and 5 parts of 1-hydroxycyclohexylphenylketone ("Irgacure 184" available from Ciba-Geigy of Hawthorne, NJ)), was stirred 0.5 g of the surface active agent, dioctyl sodium sulfosuccinate ("Aerosol OT" available from Fisher Scientific of Fairlawn, NJ). Then water was added drop by drop until the clear solution turned cloudy, indicating the end of the microemulsion region and the onset of a macroemulsion region. This procedure was repeated for a range of monomer: surface active agent ratios up to about 65 weight percent surface active agent at which point the mixture became too viscous to stir. The weight fractions of the components were calculated and plotted to give the delineation of the clear microemulsion region shown in Fig. 1.

Example 2

This example illustrates the preparation of a porous biomosaic polymer membrane with bovine serum albumin bound at surfaces of the membrane from a mixture of acrylate monomers and aqueous protein solution. To 4.5 g of a stirred solution of 49 parts by weight of ethyltriglycolmethacrylate ("BM707", Rohm Tech Inc. of Malden, MA), 49 parts propoxylated pentaerythritoltriacylate ("Photomer 4171", Henkel Corporation) and 2 parts 1-hydroxycyclohexylphenylketone ("Irgacure 184", Ciba-Geigy) at 25° C was added 3.0 grams of dioctyl sodium sulfosuccinate ("Aerosol OT", Fisher Chemical Co.). 1.2 g of this mixture was added to a 1 dram glass vial and mixed with 0.3 g of 5% bovine serum albumin, (BSA) (Sigma Chemical Co. of St. Louis, MO) in phosphate buffered saline (PBS), pH 7.0, by shaking for about 10 seconds. (All percent values are weight-percents unless otherwise noted.) The slightly cloudy emulsion which results was poured onto about a 15x15 cm square sheet of polyester film and a second sheet of film was placed on top to spread the emulsion into a thin layer. This assembly was then polymerized by repeated passage through a UV processor (Model "IC120244 AN IRDC", RPC Industries of Plainfield, IL) operating at 15.24 meters per minute using two 31 watt/cm² lamps. To ensure substantially complete polymerization, 20 passes were made on each side. The resulting opaque assembly was washed in 250 ml of methanol for 20 minutes, during which time the polyester sheets were removed. A second methanol wash was followed by 2 washes in 500 ml each of water and air drying to a white, flexible, porous biomosaic polymer membrane.

Example 3

This example illustrates the assay method used to determine the total amount of protein bound at surfaces of a biomosaic polymer membrane made by the method of Example 2. A paper punch was used to produce disk samples of the membrane, about 0.7 cm in diameter and 0.2 to 0.7 mm thick, which were then wetted with 50 µl of water. BCA Protein Assay Reagent (Pierce Chemical Company of Rockford, IL), 1 ml, was added and the mixture incubated at room temperature for two hours. After removing the membrane disk, the absorbance of the solution was read at 562 nm. This value was compared to a standard curve prepared concurrently using BSA standards. Samples of a membrane prepared as in Example 2 with water alone instead of BSA solution were also assayed by this method and were found to have no detectable absorbance above background. Additionally samples of membrane were added to the BSA standards and the resulting standard curve was no different from the one prepared without the membrane samples. These experiments indicated that there was no interference from the membrane in the protein determination. Each

membrane was sampled in triplicate and the protein values reported as the average and standard deviation of these samples. (Due to the uneven thickness of some membranes the disk samples from a single membrane were not equivalent in mass leading to larger standard deviations than would normally be expected.)

5 Example 4

This example illustrates the method used to determine the amount of protein that was irreversibly bound at surfaces of biomosaic polymer membranes prepared by the method of Example 2. Disk samples of membranes were prepared as in Example 3 and then soaked in 200 microliters of 1% sodium dodecyl sulfate (SDS) in PBS for 1 hour at 37° C. The disks and the SDS solutions were then assayed separately with BCA Protein Reagent as described in Example 3 (50 microliter samples of the SDS solution and 1 milliliter of BCA were used). The amount of protein was calculated by comparison with a standard curve prepared with BSA standards. Each membrane was sampled in triplicate and the results reported as the average and standard deviation of these samples. Using an assay capable of detecting 0.2 µg of protein, there was no protein detected in the SDS supernate, indicating that the protein was irreversibly bound.

15 Example 5

This example shows that a range of emulsion compositions can be used to bind protein at surfaces of biomosaic polymer membranes. Using the method of Example 2, membranes whose compositions varied in the ratios of protein solution to polymerizable monomer/surface active agent mixture (P/MS), surface active agent to monomer (S/M), and monomer "Photomer 4171" to monomer "BM707" (MD/MB) as indicated in Table 1 (chosen in accord with designed experiment protocol described in Chapter 15 of A.D. Rickmers et al., "Statistics: An Introduction", McGraw-Hill, New York 1967), were prepared, washed, and dried. The protein used in preparing these membranes was 1% BSA in water, and the surface active agent used was "Aerosol OT". The concentration of photoinitiator was as in Example 2 in all emulsions. The amount of protein in each resulting membrane was then determined by the analytical methods of Examples 3 and 4 and is summarized in Table 1.

Table 1

Membrane Compositions and Amounts of Bound Protein					
Membrane	P/MS	S/M	MD/MB	Protein* (µg/disk)	Post-SDS Protein* (µg/disk)
1	0.32	0.25	0.67	7.55(0.09)	3.73(0.79)
2	0.32	0.25	1.0	7.07(0.96)	7.37(0.61)
3	0.32	0.25	1.5	4.10(1.10)	2.19(0.84)
4	0.32	0.67	0.67	7.86(1.75)	5.39(0.31)
5	0.32	0.67	1.0	13.62(2.62)	9.65(0.26)
6	0.32	0.67	1.5	10.17(1.62)	5.48(0.40)
7	0.56	0.25	0.67	2.36(0.78)	1.75(0.27)
8	0.56	0.25	1.0	5.68(0.68)	3.77(0.09)
9	0.56	0.25	1.5	4.98(0.92)	3.68(0.44)
10	0.56	0.67	0.67	8.43(0.35)	7.15(1.27)
11	0.56	0.67	1.0	12.05(0.70)	10.88(0.83)
12	0.56	0.67	1.5	10.96(2.32)	11.89(0.48)
13	0.43	0.43	1.0	12.49(1.53)	9.12(1.76)
14	0.14	0.67	1.0	9.18(3.82)	8.14(4.09)
15	0.14	1.0	1.0	5.18(0.64)	4.23(0.82)
16	0.14	1.5	1.0	11.32(1.91)	8.45(0.50)
17	0.22	0.67	1.0	11.86(1.23)	12.50(1.18)
18	0.22	1.0	1.0	12.09(2.59)	10.27(3.00)
19	0.22	1.5	1.0	12.18(1.27)	10.05(1.59)
20	0.32	0.67	1.0	18.82(2.32)	15.32(1.36)
21	0.32	1.0	1.0	17.95(1.50)	13.55(0.22)
22	0.32	1.5	1.0	19.14(1.18)	15.64(1.86)

* Standard deviations are shown in parentheses.

Membranes 1, 2, 3, 7, 9, 14, and 16 in Table 1 were found to be more stiff and brittle than the others. This may be due to the low ratio of surface active agent to monomer (membranes 1, 2, 3, 7, and 9) or low protein concentration (membranes 14 and 16) present in the emulsion.

In all formulations protein was found to be bound at surfaces of the biomosaic polymer membrane. On an area basis, this ranged between 8.35 and 67.70 $\mu\text{g/square centimeter}$ of the membrane. The lowest levels occurred at low S/M ratios and the highest at high S/M ratios. It is also readily evident that the levels of protein increase as the amount of protein solution incorporated (P/MS) is increased.

The monomer ratio (MD/MB) has only a slight effect on protein binding, but can change the physical properties and durability of the membrane as indicated above. In these formulations a MD/MB ratio of 1.0 was found to be most acceptable. The amount of protein found to be resistant to SDS treatment was nearly equal to that of the untreated membranes in most cases. (Differences in membrane thickness could account for excess protein in either the treated or untreated membranes.) In no case was protein found in the SDS wash solution of these membranes.

Example 6

This example shows that the monomers used to prepare biomosaic polymer membranes can be selected from a wide range of mono-, bi-, tri-, and tetra-functional acrylates. Membranes were prepared from 48 parts of acrylate monomer or 1/1 mixtures by weight of comonomers containing 2% of "Irgacure 184", 36 parts of octylphenoxypolyethoxyethanol ("Triton X-100", Rohm and Haas) and 16 parts of 5% BSA in water by the method of Example 2. The monomers used were methyl methacrylate (MMA; "M5,590-9" Aldrich Chemical Co. of Milwaukee, WI), isobornylacrylate (IBA; "QM589" Rohm and Haas), ethyltriglycolmethacrylate ("BM707"), propoxylated glycoldiacrylate ("Photomer 4083", Henkel Corporation), hexanedioldiacrylate ("Photomer 4017", Henkel Corporation), tripropyleneglycoldiacrylate ("Photomer 4061", Henkel Corporation), aromatic diacrylate ("Photomer 4028", Henkel Corporation), propoxylated pentaerythritoltriacylate ("Photomer 4171", Henkel Corporation), trimethylolpropanetriacylate (TMPTA; "24,680-8" Aldrich Chemical Co.), or di-trimethylolpropane tetraacrylate ("Sartomer-335", Sartomer Chemical Corp. of West Chester, PA). After washing and drying the membranes, samples were assayed for protein as in Example 3. The results are shown in Table 2.

Table 2

Membrane Compositions and their Bound Protein			
Membrane	Monomer	Comonomer	Protein Found (mg/g of membrane)
1	MMA		DNC*
2	MMA	Photomer 4083	5.78 (0.56)
3	MMA	Photomer 4171	1.83 (0.62)
4	MMA	Sartomer-335	1.95 (1.22)
5	IBA		ND**
6	IBA	Photomer 4083	ND
7	IBA	Photomer 4171	0.14 (0.08)
8	IBA	Sartomer-335	ND
9	BM707		DNC
10	BM707	Photomer 4083	1.96 (0.28)
11	BM707	Photomer 4171	5.93 (0.41)
12	BM707	Sartomer-335	4.89 (0.01)
13	BM707	TMPTA	2.28 (0.51)
14	BM707	Photomer 4028	7.77 (2.41)
15	Photomer 4083		6.66 (1.79)
16	Photomer 4017		2.92 (0.39)
17	Photomer 4017	Photomer 4083	2.00 (0.70)
18	Photomer 4017	Photomer 4171	2.15 (0.35)
19	Photomer 4017	Sartomer-335	1.69 (0.53)
20	Photomer 4017	TMPTA	0.74 (0.07)
21	Photomer 4017	Photomer 4028	0.42 (0.21)

* DNC = The monomer dispersion did not polymerize under these experimental conditions.

** ND = No protein was detected.

Table 2 (continued)

Membrane Compositions and their Bound Protein			
Membrane	Monomer	Comonomer	Protein Found (mg/g of membrane)
22	Photomer 4061		8.13 (0.52)
23	Photomer 4061	Photomer 4083	2.28 (0.27)
24	Photomer 4061	Photomer 4171	1.44 (0.33)
25	Photomer 4061	Sartomer-335	3.61 (0.87)
26	Photomer 4061	TMPTA	1.09 (0.28)
27	Photomer 4061	Photomer 4028	1.88 (0.52)
28	Photomer 4028		6.17 (1.46)
29	Photomer 4028	Photomer 4083	2.89 (0.28)
30	Photomer 4028	Photomer 4171	3.64 (0.46)
31	Photomer 4028	Sartomer-335	0.74 (0.23)
32	Photomer 4171		7.92 (0.82)
33	TMPTA		0.97 (0.21)
34	Sartomer-335		1.11 (0.29)

All of the monomers used to prepare membranes in this example polymerized to an opaque white membrane. When used without a comonomer MMA and "BM707" did not fully polymerize under these conditions resulting in a gummy oil which could not be evaluated. The handling properties of the product membranes varied as would be expected with increased hardness or brittleness correlating with increased amounts of crosslinking, i.e., the incorporation of greater acrylate functionality. Some protein was detected with at least one formulation of each monomer used, varying from traces to nearly 9 mg of BSA per gram of membrane.

Example 7

This example shows that biomosaic polymer membranes can be prepared with nonionic or ionic surface active agents and that the amount of protein bound at surfaces of these membranes is influenced by the type of surface active agents. Membranes were prepared as in Example 2 using 1% BSA solution and an anionic surface active agent, "Aerosol OT" or a nonionic surface active agent, "Triton X-100", (octylphenoxypolyethoxyethanol, Rohm and Haas), "Igepal CO-850" (nonylphenoxypolyethoxyethanol, GAF Corporation of New York, NY), "Pluronic L-81" (polyethoxy-polypropoxyethanol, BASF Wyandotte Corporation of Wyandotte, MI), or "Pluradot HA410" (Polyoxyalkylene glycol, BASF Wyandotte Corporation). Membranes were also prepared with a cationic surface active agent DDAB (didodecyl-dimethylammonium bromide, Fisher Chemical Co.) or "Emcol CC9" (Polypropoxy quaternary ammonium chloride, Witco Corporation of New York, NY) from a mixture of 55 parts of a 1/1 monomer mixture of "BM707" and "Photomer 4171" with 2% "Irgacure 184", 35 parts of cationic surface active agent and 10 parts of 5% BSA. The amount of protein was determined by the method of Example 3 and is shown in Table 3.

Table 3

Surface Active Agent Used and Amounts of Bound Protein		
Membrane	Agent	Protein Found (mg/g membrane)
1	Aerosol OT	1.21
2	Triton X-100	1.47
3	Igepal CO-850	1.00
4	Pluronic L-81	2.27
5	Pluradot HA410	2.37
6	DDAB	1.38
7	Emcol CC9	4.51

These data show that membranes prepared with different types of surface active agents all contain protein and the amount of protein can be changed considerably (over 4-fold) by the choice of surface active agent.

Example 8

This example shows that the amount of protein bound at surfaces of a biomosaic polymer membrane depends upon the protein concentration in the hydrophilic liquid of the emulsion. Membranes were prepared as indicated for membrane 5 of Example 5 using 0.0, 0.25, 1.0, and 5.0% of human serum albumin (HSA, Sigma Chemical Co.) in water. As the concentration of protein in the water increased, the dispersion became somewhat cloudy. The membranes were then assayed as in Example 3 and were found to contain 0.0, 0.2, 0.95, and 3.7 mg of HSA per g of membrane, respectively. This indicates that the concentration of protein present in the hydrophilic liquid of the emulsion affects the amount of protein bound at surfaces of the membrane product.

Example 9

This example illustrates the preparation of a porous, biomosaic polymer membrane having biologically active material bound at surfaces thereof, supported on a non-reactive support. A slightly cloudy emulsion was prepared by shaking together 9.12 grams of the monomer mixture described in Example 2 and 2.88 grams of a 5% aqueous solution of human serum albumin in a 28.3g (1 ounce) bottle for about 10.0 seconds. A 10 cm by 10 cm piece of non-woven polyester sheet weighing 2 milligrams per square centimeter was then dip coated with the emulsion in a dish and placed between two sheets of polyester film, polymerized with ultraviolet light and washed as in Example 2. The resulting material was a porous membrane supported on an embedded non-woven matrix.

The amount of protein present, assayed by the method of Example 3, was 10.29 mg/gram of membrane. When prepared as a membrane without the non-woven sheet, considerably less human serum albumin, 2.91 mg/gram of membrane, was found to be present. The inclusion of a non-woven sheet not only increased the apparent strength of the product, but also increased the amount of protein.

Example 10

This example shows that the ionic strength of the protein in the hydrophilic liquid influences the amount of protein bound at surfaces of the biomosaic polymer membrane. Membranes were prepared as in Example 2 using aqueous solutions of 5% BSA in water, in PBS, or in 2 M ammonium sulfate and with "Triton X-100" or "Pluradot HA410". The emulsions which contained PBS were somewhat cloudy while those with ammonium sulfate did not polymerize under these conditions. After washing and drying, the amount of protein found by the method of Example 3 in the water membranes was 1.47 and 2.37 mg/g while the PBS membranes had 1.86 and 4.23 mg/g of membranes, respectively. This indicates that more protein was bound from the PBS solution than the other hydrophilic solutions, and that the ionic strength of the the amount of protein so bound.

Example 11

This example shows that much of the available solution protein can be bound at surfaces of the biomosaic polymer membranes. Membranes were prepared as in Example 2 using albumin labeled with radioactive carbon-14 or immunoglobulin G (IgG) labeled with radioactive carbon-14 each diluted with 5% unlabeled BSA. Analysis of the radioactivity present in the product membranes indicated that 34% of the albumin and 47% of the IgG were bound. The radioactive label also showed that in both cases about 1% of the total membrane mass consisted of protein. This indicates that a significant proportion of the available protein can be bound at surfaces of these membranes.

Example 12

This example shows that proteins can be bound with or without the use of carrier proteins. Membranes were prepared as in Example 2 using Protein A (available from Genzyme Corporation of Boston, MA) or goat antibody to human IgE (available from Immunosearch of Emeryville, CA) both labeled with I-125 using "Iodobeads™" (available from Pierce Chemical Co.), in 5% BSA or 0% BSA solution. Analysis of the radioactivity remaining in the polymerized, washed and dried membranes showed that 11% of the Protein A and 16% of the antibody were bound when carrier BSA was used. In the absence of carrier BSA the amounts of bound Protein A and antibody increased to 21 and 31%, respectively, despite a large decrease in the total available protein. These data additionally show that the binding of each protein is influenced more by its own properties than that of other proteins present.

Example 13

This example shows that DNA can be bound. Membranes were prepared by the method of Example 2 where the

aqueous solution contained 0.16 and 0.32 μ g of radiolabeled (P-32) DNA and 5% BSA. The DNA preparation was the digestion product of Hae III restriction nuclease on plasmid pBR322. The reaction mixture was then dialyzed to remove low molecular weight fragments, resulting in a solution of 200-600 base pair chains. The membranes produced were determined to contain 24% (38 ng) and 17% (54 ng), respectively, of the DNA initially present in the formulation, by counting the residual radioactivity. These data indicate that DNA can be easily bound.

Example 14

This example shows that active enzymes can be bound at surfaces of biomosaic polymer membranes prepared from emulsions containing aqueous solutions of the enzyme. Membranes were prepared with aqueous solutions of 0.025% horseradish peroxidase (HRP) ("Type VI", Sigma Chemical Co.) in 5.0, 2.5, 1.0, 0.25% and 0.0% BSA, PBS as in Example 2. After washing and drying weighed samples of the membranes were assayed for protein content as in Example 3 and for enzyme activity by the method of Doellgast and Rothberger, *Analytical Biochemistry* 147, 529 (1985) (reported as absorbance increase at 490 nm per minute per mg of membrane). The results of these determinations are shown in Table 4. It is apparent that the retention of enzyme activity is not dependent upon a carrier protein such as BSA since significant activity is recovered without it in the formulation. However, the highest enzyme activity was recovered at an intermediate level of BSA, indicating that some carrier is useful.

Table 4

Retained Horseradish Peroxidase Activity in Membranes		
BSA (Initial %)	Protein (mg/g)	HRP Activity (A490/min/mg of membrane)
0.0	ND *	0.007 (0.001)
0.25	0.42 (0.11)	0.156 (0.088)
1.0	1.34 (0.05)	0.111 (0.015)
2.5	2.65 (0.11)	0.215 (0.080)
5.0	3.58 (0.78)	0.079 (0.023)

* ND = None detected

Example 15

This example shows that alkaline phosphatase can be bound at surfaces of these biomosaic polymer membranes. Membranes were prepared as in Example 2 where the aqueous solution contained 0.025% alkaline phosphatase ("Type VII-T", Sigma Chemical Co.) and 1.0 mM magnesium chloride and zinc chloride in 1% BSA in water and the surface active agent was "Aerosol OT", "Triton X-100", "Pluronic LB1", "Pluradot HA410", or "Igepal CO850". The retained enzyme activity was assayed with p-nitrophenylphosphate by the Sigma method (Sigma Chemical Co., 1988 catalog page 1139). The results are shown in Table 5.

Table 5

Retained Alkaline Phosphatase Activity in Membranes	
Surface Active Agent	Phosphatase Activity (A410/hour/mg of membrane)
Aerosol OT	0.0038
Triton X-100	0.0742
Pluronic LB1	0.0139
Pluradot HA410	0.0073
Igepal CO850	0.0391

The 20-fold range of recovered enzyme activity found in these membranes may be due to a combination of the influence of the surface active agent interaction of the protein and monomer emulsion phases and inhibition of the enzyme by the surface active agent preparation.

Example 16

This example shows that proteins other than BSA can be substituted as a carrier protein. Membranes were prepared with aqueous solutions of pig skin gelatin, 1% in PBS (Sigma Chemical Co.); casein, saturated in PBS solution

(Sigma Chemical Co.); or human hemoglobin, saturated in PBS solution (Worthington Biochemical Co. of Malvern, PA) and 0.025% horseradish peroxidase as in Example 12. Enzyme activities, determined as in Example 14, were 0.010, 0.156, and 0.050 A490/min/mg of membrane, respectively. This indicates that other proteins can be used as a carrier for biologically active molecules.

Example 17

This example demonstrates the usefulness of antigen bound at surfaces of biomosaic polymer membranes. A membrane was prepared as in Example 2 from an aqueous solution containing 0.05% perennial ryegrass (PRG) pollen extract (3M Diagnostic Systems of Santa Clara, CA) in 1% BSA in water. The surface active agent used in this membrane was "Aerosol OT". Paper punch samples of this membrane were placed into the wells of a microtiter filtration plate with a nitrocellulose membrane bottom (Millipore Corporation of Bedford, MA) which had previously been wetted with 100 μ l of 20% methanol in PBS, followed by 200 μ l of PBS, and incubation with three 100 μ l portions of 1% BSA in PBS, each for five minutes. (Solutions added to the membrane wells were drawn through by vacuum.) The biomosaic polymer membranes were treated with 100 μ l of 1% HSA in PBS for 5 minutes, and then the solutions were drawn through the membrane by applying a vacuum.

FluoroFAST™ kit "D" calibrator (available from 3M Diagnostic Systems) was diluted with 1% human serum albumin (HSA) in PBS such that final concentrations of IgE antibody to PRG were 20, 10, and 5 IUs per ml. Aliquots, 100 μ l each, of these solutions were incubated in the wells for 30 minutes at ambient temperature followed by thorough washing with Specific IgE FAST™ Wash Buffer (3M Diagnostic Systems). The membranes were then incubated for an additional 30 minutes with 100 μ l of Specific Anti-IgE-Enzyme Conjugate (3M Diagnostic Systems) in Specific IgE FAST™ Conjugate Buffer followed by a repeat of the washing procedure. The membranes were then assayed for bound enzyme conjugate with Specific IgE FAST™ Substrate solution (3M Diagnostic Systems). After 30 minutes of incubation one set of the solutions was transferred to clean black polystyrene wells in a microtiter plate and its fluorescence read using the FluoroFAST™ microtiter well fluorometer (3M Diagnostic Systems).

The procedure was repeated with another set after 60 minutes of incubation. Fig. 2 shows that the fluorescence response increases nearly linearly with the concentration of PRG-specific IgE present in the sample solutions after 30 minute (line A) and 60 minute (line B) incubations. This response indicates that the antigenic activity of the extract was retained and that such membranes can be used to determine the amount of an antibody present in serum samples.

Example 18

This example demonstrates the usefulness of antibody biomosaic polymer membranes. A membrane was prepared as in Example 2 from an aqueous solution containing 0.05% sheep anti-human IgG antibody (available from Cooper-Biomedical of Malvern, PA) and 1% BSA in water. The surface active agent used in this preparation was "Aerosol OT". Paper punch samples of this membrane were placed in the wells of a microtiter filtration plate pretreated as in Example 17. The membranes were then wetted with 100 μ l of 1% BSA in PBS for 5 minutes and the solution was drawn through the membrane under vacuum. A solution of 300 μ g/ml of human IgG and 5 μ g/ml of human IgG conjugated to HRP (both from CooperBiomedical) in 1% BSA was diluted to final concentrations of 102, 34, 11, and 4 μ g/ml with 1% BSA. Aliquots, 100 μ l each, of these solutions were added to the membranes and allowed to incubate for 5 minutes before being drawn through. Each well and membrane was then thoroughly washed with 0.1% solution of "Tween 20" in PBS. ("Tween 20" surface active agent is available from Sigma Chemical Co.). The bound HRP was assayed for 10-15 minutes as in Example 14. The absorbances of the resulting solutions were read by transferring them to a clear flat-bottom polystyrene microtiter plate and measuring their 490 nm absorbance on a Dynatech microplate reader (Dynatech Corporation of Chantilly, VA). Fig. 3 shows that there is a nearly linear correlation of absorbance to IgG concentration shown in line A. This example shows that the biomosaic polymer membrane can be used to determine the amount of antigen in a biological sample.

Example 19

This example shows that the protein bound during polymerization is overwhelmingly congregated at the surfaces of the biomosaic polymer so formed. Two membranes were prepared by the method of Example 2 with the emulsion components and weight fractions being that for membrane 5 of Table 1, except that one emulsion's hydrophilic liquid contained no human serum albumin and the other emulsion's hydrophilic liquid contained 5.0% human serum albumin (HSA). The membranes prepared were analyzed by x-ray photoelectron spectroscopy (XPS) using a Hewlett-Packard Model "5950B" ESCA Spectrometer machine (available from Hewlett-Packard, of Palo Alto, CA) to determine the elemental content of the surface of the membrane to a depth of from at least 4nm (40 Angstroms) to as much as 10nm (100 Angstroms).

The spectrometer was set according to the instructions contained in its operations manual except as follows: a concentric hemispherical analyzer and a flood gun set at 2-4 milliamperes and 4-5 electron volts were used; an 800 watt monochromatic x-ray beam using Al-K-alpha x-rays was used; and as a reference, the carbon 1s signal at 285 electron volts was used.

Since the only source of elemental nitrogen in these membranes is the protein, the level of nitrogen found by XPS indicated the amount of protein bound at surfaces of the biomosaic polymer membranes. Table 6 shows the results obtained.

Table 6

Membrane HSA (%)	XPS Elemental Analysis (%)		
	Carbon	Oxygen	Nitrogen
0.0	72	28	0.0
5.0	71	27	2.3

The magnitude of the elemental nitrogen found by XPS analysis of the surface indicated that an unexpectedly large amount of protein was bound there.

Nitrogen comprises only about 14.3% of the non-hydrogen atoms of protein. An emulsion having a protein content of 1.6% (5% HSA times 0.32 P/MS) had only about 0.2% nitrogen. If all of the emulsion's protein were combined with the hydrophobic polymerizable monomer during polymerization, and if the protein were evenly distributed throughout the mass of the polymer, the XPS surface elemental analysis would also show 0.2% nitrogen. Ten times that amount was unexpectedly measured at the surface of the biomosaic polymer membrane. Thus, the protein was congregated overwhelmingly at the surface of the biomosaic polymer.

If the depth of penetration of the x-ray analysis were limited to the minimum of about 4nm (40 Angstroms), about the longest dimension of HSA, the percentage of the surface area of the polymer comprising biologically active material would be calculated to be at least about 16%, or the ratio of measured elemental nitrogen found at the surface to the total elemental nitrogen theoretically possible at the surface: 2.3/14.3.

As seen in Example 11, about 34% of the albumin was found to be bound. If the efficiency of binding in this Example were the same as that found in Example 11, and if the efficiency of binding could then be increased to as much as the 90% believed possible according to the present invention, then the surface area of the polymer comprising the biologically active material could be at least as much as about 42% of the total surface area.

It is known that the depth of penetration of the x-ray analysis depends on the material's properties and the power of the x-ray beam. If the depth of penetration were greater than 4 nm (40 Angstroms) (as is known) to frequently occur at least twice as far, e.g., to a depth of 8nm (80 Angstroms) (well within the state of the art) then the theoretical percentage of nitrogen would at least be halved, and percentage surface area of biologically active material previously calculated in this Example would at least double from 16% to 32% and from 42% to 84%.

While embodiments of the invention and examples have been disclosed, the scope of the invention is not limited to them but found in the claims below.

Claims

1. A porous, biomosaic, polymeric membrane, said membrane comprising at least a minimally useful amount of at least one biologically active material irreversibly bound at surfaces of said membrane, wherein said membrane has pores from 0.01 micrometers to 10 micrometers, and wherein said membrane is a polymer formed from microemulsion of at least one hydrophobic polymerizable monomer polymerized in the presence of said biologically active material.
2. The membrane, according to Claim 1, wherein said biologically active material is congregated at said surfaces of said membrane and comprises at said surfaces between as little of said surfaces as is minimally useful to as much of said biologically active surfaces as 84 percent, said biologically active material comprising a biochemically active or reactive material, an immunochemically active or reactive material, a physiologically active or reactive material, a pharmaceutically active or reactive material, or combinations thereof.
3. The membrane according to any preceding claim wherein said biologically active material comprises an amino acid, a carbohydrate, a lipid, a nucleic acid, a protein, an antibody, an antigenic substance, an enzyme, a co-factor, an inhibitor, a lectin, a hormone, a receptor, a coagulation factor, a growth enhancer, a histone, a vitamin, a drug,

a cell surface marker, a herbicide, a pesticide, or combinations thereof.

4. The use of a membrane according to any preceding claim for immunoassay, as an enzyme reactor, as a bioseparator, or combinations thereof.

5. A process for preparing a porous, biomosaic, polymeric membrane wherein said polymer is prepared by polymerization at no lower than ambient temperature of a water-in-oil or a bicontinuous microemulsion of a hydrophobic polymerizable monomer, a biologically active material, at least one hydrophilic liquid, and at least one surface active agent;

wherein polymerization comprises mixing said microemulsion, dispensing said microemulsion to form the polymeric membrane, and polymerizing said microemulsion at ambient temperature and with high energy radiation to polymerize said hydrophobic polymerizable monomer to form a biomosaic porous polymeric membrane having said biologically active material bound at surfaces of said polymer.

6. The process according to Claim 5, wherein said hydrophobic polymerizable monomer comprises an addition-polymerizable unsaturated organic compound having at least one double bond between two carbon atoms in said microemulsion in an amount of from 1 to 97 weight percent of said microemulsion;

wherein said surface active agent comprises ionic surface active agents, nonionic surface active agents, zwitterionic surface active agents, or combinations thereof in said microemulsion in an amount of from 2 weight percent to 97 weight percent of said microemulsion; and

wherein said biologically active material is in said hydrophilic liquid and the amount of said biologically active material in said hydrophilic liquid is less than saturation amount, wherein said hydrophilic liquid comprises of from 1 to 97 weight percent of said microemulsion, and wherein from 1 percent to about 90 percent of said biologically active material in said microemulsion during said polymerization of said hydrophobic polymerizable monomer is bound at said surfaces of said polymer after polymerization.

7. The process according to Claim 6, wherein said organic compound comprises a monofunctional acrylate, a multifunctional acrylate, or combinations thereof in said microemulsion in an amount of from 25 to 80 weight percent of said microemulsion;

wherein said ionic surface active agents comprise salts of carboxylic acids having a carbon chain length of from 8 to 22 atoms, sulfonic acids, sulfuric acid esters, phosphoric acid esters, polyphosphoric acid esters, quaternary ammonium salts, oxides or salts of amine compounds having a carbon chain length of from 8 to 22 atoms, or combinations thereof;

wherein said nonionic surface active agents comprise polyoxyethylenated-alkyl phenols, polyoxyethylenated-straight chain alcohols, polyoxyethylenated-polypropylene glycols, carboxylic acid esters having a carbon chain length of from 8 to 22 atoms, or combinations thereof;

wherein said zwitterionic surface active agents comprise beta-N-alkylaminopropionic acids, N-alkyl-beta-imidopropionic acids, imidazoline carboxylates, N-alkylbetaines, sulfobetaines, or sultaines.

8. The process according to Claim 7, wherein said organic compound comprises isooctyl acrylate, isobornyl acrylate, 2-ethylhexyl acrylate, ethylenediglycol acrylate, ethyltriglycol methacrylate, 1,6-hexanediol diacrylate, tetraethyleneglycol diacrylate, tripropyleneglycol diacrylate, propoxylated glycol diacrylate, pentaerythritol triacrylate, trimethylolpropane triacrylate, pentaerythritol tetraacrylate, di-trimethylolpropane tetraacrylate or combinations thereof and wherein from 5 percent to 75 percent of said biologically active material in said microemulsion during said polymerization of said hydrophobic polymerizable monomer is bound to said surfaces of said polymer after said polymerization.

9. The process according to any preceding claim wherein the membrane has pores of from 0.01 micrometers to 10 micrometers and wherein as little of said biologically active material as is minimally useful to as much of said biologically active material as 70 mg/cm² of said membrane is bound at surfaces of said polymer.

Patentansprüche

1. Poröse Biomosaik-Polymermembran, umfassend mindestens eine minimal verwendbare Menge von mindestens einem biologisch aktiven Material, das an Oberflächen der Membran irreversibel gebunden ist, wobei die Membran

Poren von 0,01 Mikrometern verfügt und wobei die Membran ein Polymer ist, gebildet aus Mikroemulsion von mindestens einem hydrophoben, polymerisierbaren Monomer, das in Gegenwart des biologisch aktiven Materials polymerisiert ist.

- 5 2. Membran nach Anspruch 1, bei welcher das biologisch aktive Material an den Oberflächen der Membran angesammelt ist und an den Oberflächen zwischen nicht weniger der Oberflächen ausmacht, als minimal verwendbar ist, und bis zu nicht mehr der biologisch aktiven Oberflächen als 84 Prozent, wobei das biologisch aktive Material ein biochemisch aktives oder reaktives Material umfaßt, ein immunchemisch aktives oder reaktives Material, ein physiologisch aktives oder reaktives Material oder Kombinationen davon.
- 10 3. Membran nach einem der vorgenannten Ansprüche, bei welcher das biologisch aktive Material umfaßt: eine Aminosäure, ein Carbohydrat, ein Lipid, eine Nucleinsäure, ein Protein, ein Antikörper, eine antigene Substanz, ein Enzym, einen Cofaktor, einen Wachstumsverstärker, ein Histon, ein Vitamin, ein Medikament, einen Zelloberflächen-Marker, ein Herbizid, ein Pestizid oder Kombinationen davon.
- 15 4. Verwendung einer Membran nach einem der vorgenannten Ansprüche zum Immunoassay, als ein Enzymreaktor, als ein Bioseparator oder Kombinationen davon.
- 20 5. Verfahren zum Herstellen einer porösen Biomosaik-Polymermembran, bei welchem das Polymer hergestellt wird durch Polymerisation bei nicht weniger als Umgebungstemperatur einer Wasser-in-Öl- oder einer bikontinuierlichen Mikroemulsion eines hydrophoben, polymerisierbaren Monomers, eines biologisch aktiven Materials, mindestens einer hydrophilen Flüssigkeit und mindestens eines oberflächenaktiven Mittels;
bei welchem die Polymerisation umfaßt: Mischen der Mikroemulsion; Ausbringen der Mikroemulsion zur Bildung der Polymermembran sowie Polymerisieren der Mikroemulsion bei Umgebungstemperatur und mit hoher
25 Energiestrahlung zum Polymerisieren des hydrophoben, polymerisierbaren Monomers unter Bildung einer porösen Biomosaik-Polymermembran, die das biologisch aktive Material an der Oberfläche des Polymers gebunden aufweist.
- 30 6. Verfahren nach Anspruch 5, bei welchem das hydrophobe, polymerisierbare Monomer eine additionspolymerisierbare, ungesättigte organische Verbindung mit mindestens einer Doppelbindung zwischen zwei Kohlenstoffatomen in der Mikroemulsion in einer Menge von 1 ... 97 Gewichtsprozent der Mikroemulsion umfaßt;
bei welchem das oberflächenaktive Mittel ionische oberflächenaktive Mittel, nichtionische oberflächenaktive Mittel, zwitterionische oberflächenaktive Mittel oder Kombinationen davon in der Mikroemulsion in einer Menge
35 von 2 Gewichtsprozent bis 97 Gewichtsprozent der Mikroemulsion umfaßt; und
bei welchem sich das biologisch aktive Material in der hydrophilen Flüssigkeit befindet und die Menge des biologisch aktiven Materials in der hydrophilen Flüssigkeit kleiner als die Sättigungsmenge ist, wobei die hydrophile Flüssigkeit 1 ... 97 Gewichtsprozent der Mikroemulsion ausmacht und wobei 1 Prozent bis etwa 90 Prozent des biologisch aktiven Materials in der Mikroemulsion während der Polymerisation des hydrophoben, polymerisierbaren Monomers an den Oberflächen des Polymers nach der Polymerisation gebunden sind.
- 40 7. Verfahren nach Anspruch 6, bei welchem die organische Verbindung in der Mikroemulsion ein monofunktionelles Acrylat, ein multifunktionelles Acrylat oder Kombinationen davon in einer Menge von 25 ... 85 Gewichtsprozent der Mikroemulsion umfaßt;
45 bei welchem die ionischen oberflächenaktiven Mittel umfassen: Salze von Carbonsäuren mit einer Kohlenstoff-Kettenlänge von 8 bis 22 Atomen, Sulfonsäuren, Schwefelsäureester, Phosphorsäureester, Polyphosphorsäureester, quaternäre Ammoniumsalze, Oxide oder Salze von Amin-Verbindungen mit einer Kohlenstoff-Kettenlänge von 8 bis 22 Kohlenstoffatomen oder Kombinationen davon;
50 bei welchem die nichtionischen oberflächenaktiven Mittel umfassen: polyoxyethylierte Alkylphenole, polyoxyethylierte geradkettige Alkohole, polyoxyethylierte Polypropylenglykole, Carbonsäureester mit einer Kohlenstoff-Kettenlänge von 8 bis 22 Kohlenstoffatomen oder Kombinationen davon;
bei welchem die zwitterionischen oberflächenaktiven Mittel umfassen: beta-N-Alkylaminopropansäuren, N-Alkylbeta-aminodipropansäuren, Imidazolincarboxylate, N-Alkylbetaine, Sulfobetaine oder Sultaine.
- 55 8. Verfahren nach Anspruch 6, bei welchem die organische Verbindung umfaßt: Isooctylacrylat, Isobornylacrylat, 2-Ethylhexylacrylat, Ethylendiglykolacrylat, Ethyltriglykolmethacrylat, 1,6-Hexandioldiacrylat, Tetraethylenglykoldiacrylat, Tripropylenglykoldiacrylat, propoxyliertes Glykoldiacrylat, Pentaerythritetraacrylat, Di-trimethylolpro-

pantetraacrylat oder Kombinationen davon, und worin 5 % ... 75 Gewichtsprozent des biologisch aktiven Materials in der Mikroemulsion während der Polymerisation des hydrophoben, polymerisierbaren Monomers an den Oberflächen des Polymers nach der Polymerisation gebunden sind.

- 5 9. Verfahren nach einem der vorgenannten Ansprüche, bei welchem die Membran Poren von 0,01 Mikrometer bis 10 Mikrometer aufweist und bei welchem von dem biologisch aktiven Material nicht weniger als minimal verwendbar ist und bis zu nicht mehr des biologisch aktiven Materials als 70 mg/cm² der Membran an den Oberflächen des Polymers gebunden ist.

10

Revendications

- 15 1. Membrane poreuse, biomosaïque, polymère, ladite membrane comprenant au moins une quantité minimalement utile d'au moins un matériau biologiquement actif liée de façon irréversible aux surfaces de ladite membrane, ladite membrane ayant des pores de 0,01 micromètre à 10 micromètres, ladite membrane étant un polymère formé à partir d'une microémulsion d'au moins un monomère polymérisable hydrophobe polymérisé en présence dudit matériau biologiquement actif.
- 20 2. Membrane suivant la revendication 1, dans laquelle ledit matériau biologiquement actif est rassemblé au niveau desdites surfaces de ladite membrane et correspond à une valeur desdites surfaces qui se situe entre au moins ce qui est minimalement utile desdites surfaces et au plus 84 % desdites surfaces biologiquement actives, ledit matériau biologiquement actif comprenant un matériau biochimiquement actif ou réactif, un matériau immunochemiquement actif ou réactif, un matériau physiologiquement actif ou réactif, un matériau pharmaceutiquement actif ou réactif ou un de leurs mélanges.
- 25 3. Membrane suivant l'une quelconque des revendications précédentes, dans laquelle ledit matériau biologiquement actif comprend un aminoacide, un carbohydrate, un lipide, un acide nucléique, une protéine, un anticorps, une substance antigénique, un enzyme, un co-facteur, un inhibiteur, une lectine, une hormone, un récepteur, un facteur de coagulation, un activateur de croissance, une histone, une vitamine, un médicament, un marqueur de surface cellulaire, un herbicide, un pesticide ou un de leurs mélanges.
- 30 4. Utilisation d'une membrane suivant l'une quelconque des revendications précédentes, pour immunoessai, en tant que réacteur enzymatique, bioséparateur ou leurs mélanges.
- 35 5. Procédé pour préparer une membrane poreuse, biomosaïque, polymère, dans lequel ledit polymère est préparé par polymérisation, à une température non inférieure à la température ambiante, d'une microémulsion eau-dans-huile ou bicontinue d'un monomère polymérisable hydrophobe, d'un matériau biologiquement actif, d'au moins un liquide hydrophile et d'au moins un agent tensioactif;
- 40 où la polymérisation comprend le mélange de ladite microémulsion, la distribution de ladite microémulsion pour former une membrane polymère et la polymérisation de ladite microémulsion à température ambiante et avec un rayonnement de haute énergie pour polymériser ledit monomère polymérisable hydrophobe afin de former une membrane poreuse biomosaïque polymère ayant ledit matériau biologiquement actif lié aux surfaces dudit polymère.
- 45 6. Procédé suivant la revendication 5, dans lequel ledit monomère polymérisable hydrophobe comprend un composé organique insaturé polymérisable par addition ayant au moins une double liaison entre deux atomes de carbone dans ladite microémulsion selon une quantité de 1 à 97 % en poids de ladite microémulsion ;
- 50 dans lequel ledit agent tensioactif comprend des agents tensioactifs ioniques, des agents tensioactifs non ioniques, des agents tensioactifs zwitterioniques ou leurs mélanges dans ladite microémulsion selon une quantité de 2 % en poids à 87 % en poids de ladite microémulsion ; et,
- 55 dans lequel ledit matériau biologiquement actif est dans ledit liquide hydrophile et la quantité dudit matériau biologiquement actif dans ledit liquide hydrophile est inférieure à celle de la saturation, ledit liquide hydrophile correspondant à 1 à 97 % en poids de ladite microémulsion, et une quantité de 1 à environ 90 % dudit matériau biologiquement actif présent dans ladite microémulsion durant ladite polymérisation dudit monomère polymérisable hydrophobe étant liée auxdites surfaces dudit polymère après polymérisation.
7. Procédé suivant la revendication 6, dans lequel ledit composé organique comprend un acrylate monofonctionnel,

un acrylate multifonctionnel, ou leurs combinaisons dans ladite microémulsion selon une quantité de 25 à 80 % en poids de ladite microémulsion ;

5 où lesdits agents tensioactifs ioniques comprennent des sels d'acides carboxyliques ayant une longueur de chaîne hydrocarbonée de 8 à 22 atomes de carbone, des acides sulfoniques, des esters de l'acide sulfurique, des esters de l'acide phosphorique, des sels d'ammonium quaternaires, des oxydes ou sels de composés amine ayant une longueur de chaîne hydrocarbonée de 8 à 22 atomes de carbone ou un de leurs mélanges ;
 10 où lesdits agents tensioactifs non ioniques comprennent des alkylphénols polyoxyéthylénés, des alcools à chaîne droite polyoxyéthylénés, des propylèneglycols polyoxyéthylénés, des esters d'acide carboxylique ayant une longueur de chaîne hydrocarbonée de 8 à 22 atomes de carbone, ou un de leurs mélanges ;
 où lesdits agents tensioactifs zwitterioniques comprennent des acides bêta-N-alkylaminopropioniques, des acides N-alkyl-bêta-iminodipropioniques, des imidazoline carboxylates, des N-alkylbétaines, des sulfobétaines ou des sultaïnes.

15 8. Procédé suivant la revendication 7, dans lequel ledit composé organique comprend l'acrylate d'isooctyle, l'acrylate d'isobornyle, l'acrylate de 2-éthylhexyle, l'acrylate d'éthylènediglycol, le méthacrylate d'éthyltriglycol, le diacrylate de 1,6-hexanediol, le diacrylate de tétraéthylèneglycol, le diacrylate de tripropylèneglycol, le diacrylate de glycol propoxylé, le triacrylate de pentaérythritol, le triacrylate de triméthylolpropane, le tétraacrylate de pentaérythritol, le tétraacrylate de ditriméthylolpropane, ou un de leurs mélanges, une quantité de 5 % à 75 % dudit matériau
 20 biologiquement actif présent dans ladite microémulsion durant ladite polymérisation dudit monomère polymérisable hydrophobe étant liée auxdites surfaces dudit polymère après ladite polymérisation.

9. Procédé suivant l'une quelconque des revendications précédentes, dans lequel la membrane à des pores de 0,01 micromètre à 10 micromètres, et dans lequel une quantité dudit matériau biologiquement actif, qui se situe entre
 25 au moins ce qui est minimalement utile et au plus 70 mg/cm² de ladite membrane, est liée auxdites surfaces dudit polymère.

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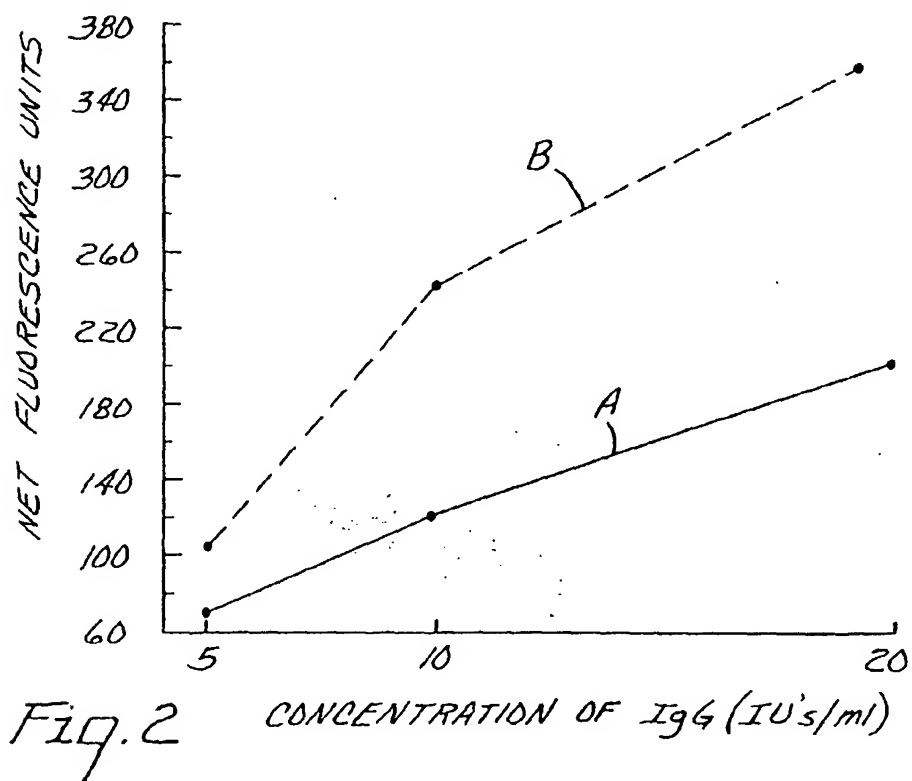
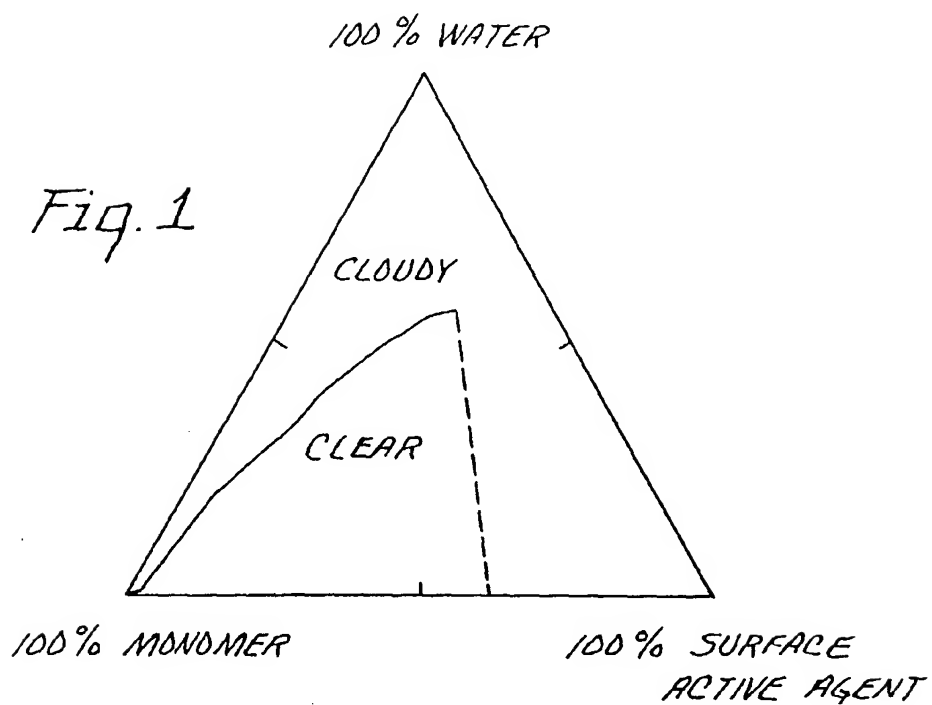
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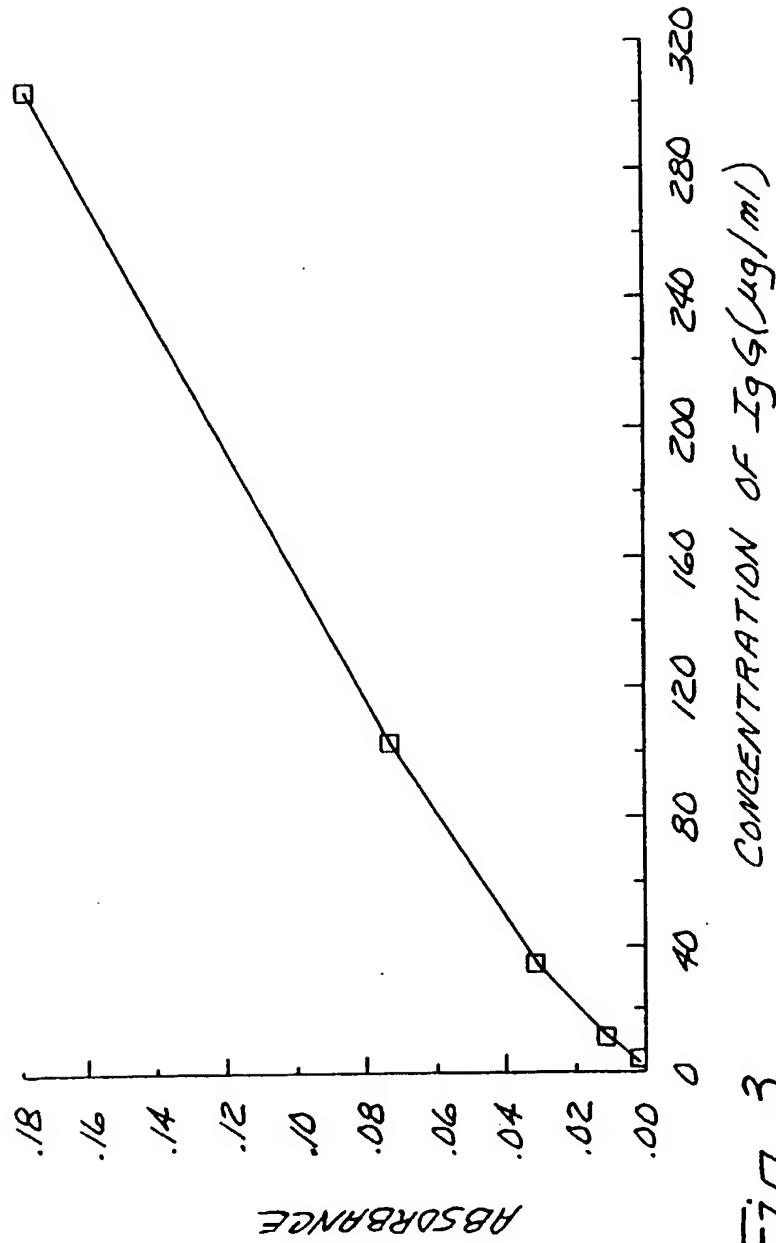


Fig. 3